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(54) Title: ENV-GLYCOPROTEIN VACCINE FOR PROTECTION OF HTLV-I AND -II INFECTION

(57) Abstract

The present invention relates to novel protein antigens derived from the HTLV env protein, that are capable of being used as a vaccine to aid in the prevention and treatment of HTLV-I and HTLV-II infections and novel methods for the production of such an antigen.

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ENV-GLYCOPROTEIN VACCINE FOR PROTECTION OF HTLV-I AND -II INFECTION

This application is a continuation-in-part of copending application Serial No. 08/681,054 filed July 22, 1996, which is incorporated by reference herein in its entirety.

1. INTRODUCTION

The present invention relates to novel protein antigens for use in a vaccine to treat and prevent human T-cell lymphotropic virus-I (HTLV-I) and HTLV-II infection, and novel methods of efficiently producing such antigens. The present invention further relates to the nucleotide sequences encoding the novel antigen and vectors and expression systems, both eucaryotic and procaryotic, to express the novel antigen. More particularly, the present invention relates to methods of producing recombinant HTLV envelope (env) glycoproteins using insect and mammalian cell lines to express useful amounts of the envelope glycoprotein.

2. BACKGROUND OF THE INVENTION

Human T-cell lymphotropic virus type I (HTLV-I) and type II (HTLV-II) are genetically and serologically related members of a group of retroviruses sharing a tropism for T lymphocytes and an association with rare lymphotropic diseases. (Hall et al., 1994, Seminars in Virology 5:165-178) HTLV-I is endemic in a number of well established geographic areas, where infection is associated with adult T cell leukemia (ATL), a malignancy of mature T lymphocytes, and a chronic encephalomyelopathy known both as HTLV-I associated myelopathy and tropical spastic paraparesis (HAM/TSP).

HTLV-I infection is endemic in southwestern Japan, the Caribbean, South America and some regions of Africa. HTLV-II infection has now been clearly shown to be endemic in a large number of native American Indian populations, and high rates of infection have also been demonstrated in intravenous drug abusers (IVDAs) in North America and Europe. (Hall et al., 1994, Seminars in Virology 5:165-178)

The vast majority of infected individuals remain as asymptomatic carriers, and serve as a source for further transmission of the virus. Although the modes of transmission of HTLV-II remain less well established than those of HTLV-I, all evidence obtained to date suggests that they are similar, if not identical. HTLV-I transmission occurs by three major routes: vertically from mother to child, which occurs primarily through breast-feeding; heterosexual and homosexual transmission; and via contaminated blood products, which may occur after blood transfusion or by intravenous drug abuse (Hollsberg et al., 1993, New England J. Medicine 328: 1173).

Over the past decade there has been accumulating evidence that HTLV-II infection may be associated with a spectrum of neurological, and possibly rare lymphoproliferative disorders. At present it is unclear if HTLV-II is less pathogenic than HTLV-I, or whether the observed lack of clinical disorders may simply reflect the comparatively small number of infected individuals so far identified, and who have been clinically evaluated.

The size and the structural organization of HTLV-II provirus has been shown to be very similar to that of HTLV-I. (Shimotono et al., 1985 Proc. Natl. Acad. Sci. USA 82:3101-3105). The similar identity of much of the primary amino acid sequence would suggest antigenic cross-reactivity between HTLV-I and HTLV-II. The genome is flanked by long terminal repeats (LTRs) which contain the binding site for the RNA polymerase, and sequences that regulate virus transcription. Four major genes have been identified and occupy the following positions in the genome LTR-gag-pol-env-pX-LTR (Seiki et al., 1983 Proc. Natl. Acad. Sci. 80:3618-3622).

The *gag* gene encodes a polyprotein which is processed to produce three internal virus structural proteins. The *pol* gene encodes reverse transcriptase, RNase H, and integrase, all of which are involved in the synthesis and integration of provirus into the host genome. An open reading frame for a

putative viral protease is located at the 3′ end of the *gag* gene. This extends into the *pol* region and is thought to be expressed from mRNA via mechanisms involving ribosomal frame shifting. (Shimotono et al., 1985 Proc. Natl. Acad. Sci. USA 82:3101-3105).

The *env* gene is located upstream of the 3′ end of the *pol* gene and partially overlaps it. The *env* gene encodes a precursor protein p63 which undergoes proteolytic cleavage, and subsequent glycosylation to produce two glycoproteins, gp46 and gp21. The gp46 protein constitutes the surface projections observed by electron microscopy on native virus particles, is believed to have receptor binding activity, and contains domains responsible for the production of neutralizing antibodies. The gp21 protein is the transmembrane glycoprotein, and by analogy with HIV may be involved in cell fusion activity. The env proteins interact with as of yet unidentified cellular receptors to mediate viral entry.

The env protein has been deduced by its nucleotide sequence to have a hydrophobic signal sequence at its amino terminus, five potential acceptor sites for N-glycosylation linked carbohydrates in the central portion, and a second cluster of hydrophobic amino acids in the putative transmembrane domain (Seiki et al., 1983 Proc. Natl. Acad. Sci. 80:3618-3622). Its sequence character suggests that it has a typical structure of a cell membrane glycoprotein.

There has been much research focused on the development of a vaccine against HTLV-I and HTLV-II. The env protein has been a target for such a vaccine, however efforts to develop a full length gp63 to use as an effective antigen have failed. Previous attempts to express useful levels of the HTLV env protein have been unsuccessful. Therefore a number of groups have instead developed synthetic peptides derived from the sequence of the env protein as antigens for HTLV-I (U.S. 5,378,805, U.S. 5,066,579) and HTLV-II (U.S. 5,378,805, U.S. 5,359,029). The primary uses of these peptides are for diagnosis of disease and the development of vaccines.

Synthetic peptides have been used increasingly to map antigenic determinants on the surface of proteins and as possible vaccines. These chemically synthesized peptides have been utilized in highly sensitive assays to distinguish between HTLV-I and -II infections and to develop vaccines (U.S. 5,476,765).

Viral vectors capable of expressing the recombinant env protein have been suggested as a vaccine for HTLV-I, such as a live adenovirus recombinant virus expressing the HTLV-I envelope protein (deThe et al., 1994, Ciba Foundation Symposium 187:47-60). The HTLV-I env protein expressed in vaccinia virus has also been formulated into a vaccine preparation (Seiki et al., 1990, Virus Genes 3:235-249; Shida et al., 1987, EMBO J. 6:3379-3384). Another group has developed a vaccine consisting of a live recombinant poxvirus expressing the full length envelope protein of HTLV-I (Franchini et al., 1995, AIDS Research and Human Retroviruses 11:307-313). However, a combination of this vaccine with two additional boosts of the gp63 protein subunit failed to confer protection suggesting that the administration of the gp63 protein subunit negated the protective efficacy of the vaccine.

The purification of the full length glycosylated gp63 has been described for use in an assay to determine the presence of anti-HTLV antibodies in a biological specimen. In the same report it is suggested that the full length glycosylated gp63 may be used in a vaccine formulation, but the efficacy of such a vaccine is not described. (U.S. 4,743,678 and U.S. 5,045,448).

Therefore there remains a need for an effective full-length HTLV env antigen to be used in vaccine formulations and an efficient means of producing such an antigen.

3. SUMMARY OF THE INVENTION

The present invention relates to novel protein antigens derived from the HTLV env protein, that are capable of being used as a vaccine to aid in the prevention and treatment of

HTLV-I and HTLV-II infections and novel methods for the production of such an antigen. The present invention relates to nucleotide sequences that encode the novel antigenic protein, mutants and derivatives thereof. The present invention further relates to methods of expressing the novel antigen, including expression vectors and cell lines, both eucaryotic and procaryotic. The invention still further relates to methods of using this novel antigen as an immunogen in vaccine preparations for the prevention and/or treatment of HTLV-I and HTLV-II infections.

The present invention relates to an HTLV env protein lacking all or a portion of its membrane spanning domain such that the polypeptide, when expressed recombinantly, is not anchored in the membrane of the host cell. In a preferred embodiment of the invention, the soluble HTLV env protein is lacking all or a portion of its amino terminus. The present invention further relates to an amino truncated form of the HTLV env protein which is soluble and accumulates in the cytoplasm of the host cell, so that the HTLV env protein is readily purified from lysed host cells.

More particularly, the present invention relates to nucleotide sequences encoding an amino terminally truncated form of the HTLV env protein, the expression of the recombinant HTLV env protein in host cell lines and the use of the resulting recombinant env protein in vaccine preparations for the prevention of HTLV infection.

A present difficulty in mammalian recombinant gene expression is that many proteins are resistant to expression in many systems, therefore the likelihood of success is difficult to predict. Previous attempts to express high levels of the HTLV env protein have been unsuccessful. The Applicant's invention has overcome this difficulty by expressing a truncated form of the HTLV env gene in a baculovirus expression system. The transcription of a cDNA corresponding to an amino terminally truncated form of the HTLV env protein led to an unexpected abundance of transcribed protein. It was found that an approximate

50 fold increase (relative to expression of the full length gene in mammalian cells) in the expression of an immunologically useful HTLV-1 env protein could be achieved.

The invention is further based on the Applicant's discovery that an antigenic protein having the amino acid sequence of the HTLV-I or the HTLV-II env protein with the amino terminal leader or signal sequence deleted, serves to protect the recipient when challenged with an inoculation of HTLV-I or HTLV-II. The immunogenicity of this protein is unexpectedly strong.

In a preferred embodiment of the invention, the nucleotide sequences encoding an amino terminally truncated form of HTLV env protein, upon expression in an appropriate host cell, produce a polypeptide that is antigenic or immunogenic. Antigenic polypeptides are capable of being immunospecifically bound by an antibody to the antigen. Immunogenic polypeptides are capable of eliciting an immune response to the antigen, e.g., when immunization with the polypeptide elicits production of an antibody that immunospecifically binds the antigen or elicits a cell-mediated immune response directed against the antigen.

In another preferred embodiment of the invention, the antigen protein of the present invention is expressed in a baculovirus system to produce an unglycosylated antigen or the antigen protein is expressed in a stably transfected T cell line to produce a glycosylated antigen. In yet another preferred embodiment of the invention the amino terminally truncated HTLV env protein is expressed as a fusion protein in order to facilitate purification of the protein.

In another aspect of the invention, methods of using these novel antigenic proteins are described. These methods include using these novel antigenic proteins in vaccine preparations in a solely preventative way, and/or in a therapeutic procedure after the recipient is already infected with either HTLV-I or HTLV-II, or both. The novel antigenic proteins of the invention also have utility in diagnostic

immunoassays, passive immunotherapy, and generation of antiidiotypic antibodies.

3.1. DEFINITIONS

As used herein, the following terms will have the meanings indicated.

The term "gp63" refers to the 63 kilodalton precursor protein of the outer membrane protein or env protein of the HTLV-I or -II virus. The term also refers to mutants, variants or fragments of gp63.

The term "gp46" refers to 46 kilodalton outer membrane protein or env protein of HTLV-I or -II virus. The term also refers to mutants, variants or fragments of gp46.

The term "env protein" refers to polypeptides comprising the native sequence of the HTLV-I and/or -II env protein, full-length and truncated, as well as analog thereof. Preferred analogs are those which are substantially homologous to the corresponding native amino acid sequence, and most preferably encode at least one native HTLV-I and -II env epitope, such as a neutralizing epitope. A more preferred class of HTLV-I and -II env polypeptides are those lacking a sufficient portion of the C-terminal transmembrane domain to promote efficient expression and/or secretion of the HTLV-I and II env proteins at high levels from insect or mammalian cell expression hosts of the present invention.

The term "effective amount" refers to an amount of HTLV-I and -II env polypeptide sufficient to induce an immune response in the subject to which it is administered. The immune response may comprise, without limitation, induction of cellular and/or humoral immunity.

The term "treating or preventing HTLV infection" means to inhibit the replication of the HTLV virus, to inhibit HTLV transmission, or to prevent HTLV from establishing itself in its host, and to ameliorate or alleviate the symptoms of the disease caused by HTLV infection. The treatment is considered therapeutic if there is a reduction in viral load, decrease in mortality and/or morbidity.

The term "pharmaceutically acceptable carrier" refers to a carrier medium that does not interfere with the effectiveness of the biological activity of the active ingredient, is chemically inert and is not toxic to the patient to whom it is administered.

The term "therapeutic agent" refers to any molecule compound or treatment, preferably an antiviral, that assists in the treatment of a viral infection or the diseases caused thereby.

4. BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. The nucleotide sequence and the amino acid sequence of the HTLV-I env protein. The boxed portion of the sequence corresponds to those sequences which are deleted in one embodiment of the amino terminally truncated form of the HTLV-I env antigen of the present invention.

FIGURE 2. The nucleotide sequence and the amino acid sequence of the HTLV-II env protein. The boxed portion of the sequence corresponds to those sequences which are deleted in one embodiment of the amino terminally truncated form of the HTLV-II env antigen of the present invention.

FIGURE 3. Detection of the HTLV-II recombinant env glycoprotein of 63 kDa (rgp63) expressed in the H5 cells by western blotting using HTLV-II infected human serum as the specific antibody (Lane 1). The protein was not detected in non-infected insect cells (Lane 2). HTLV-II negative human serum did not react with the HTLV-II env glycoprotein expressed in insect cells (Lane 3).

FIGURE 4. Detection of antibody against env gp46 1 week after immunization of R3 and R4 with rgp63 (lane 2 and 3, respectively). The antigen used in the detection system was a GST HTLV-II gp46 fusion protein. The serum of the R3 prior to immunization did not react with the antibody to the GST-gp46 fusion protein (lane 1).

FIGURE 5. FACS analysis of HTLV-II infected cell lines using immunized rabbit, R3. Positive staining was detected in the cell lines, Vines and Mo-T. In contrast CEM was negative. Dotted line: control FITC labeled anti-rabbit antibody. Solid line anti HTLV-II env protein rabbit serum used at a 1:10 dilution. Fl - fluorescence intensity.

FIGURE 6A. Antibody titers of the animals after inoculation of HTLV-II-Vines examined by particle agglutination (PA) method. The left figure (A) shows the antibody titers of non-immunized rabbit. The right figure (B) shows the antibody response in rabbits preimmunized with the recombinant gp63. Closed circle : R1, open circle: R2, open square : R3, closed square : R4.

FIGURE 6B. Detection of antibody against GST-gp46 of R1 after inoculation of HTLV-II Vines cells. Antibody was first detected after 2 weeks and was present. The antigen used in the detection system was a GST-gp46 fusion protein transferred to a nylon membrane.

FIGURE 7. Detection of HTLV-II provirus DNA by southern hybridization of nested PCR.
Number denotes the week after inoculation. Only alive HTLV-II-Vines cells-injected Rabbits (Rl, R2 and R5) showed positivity after long time. In contrast, vaccinated animals (R3, R4, R8 and R9) or injected with heat inactivated HTLV-II-Vines cells (R6 and R7) showed a negative response.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel antigenic protein derived from the HTLV env protein, that can be used as an immunogen in a vaccine preparation to aid in the prevention and treatment of HTLV-I and HTLV-II infections. The invention also relates to methods for the production of such an antigen.

The present invention relates to an HTLV env polypeptide lacking all or a portion of its membrane spanning domain, such that the polypeptide when expressed recombinantly is not anchored in the membrane of the host cell. In a preferred embodiment of the invention, the soluble HTLV env protein is lacking all or a portion of its amino terminus. The present invention further relates to an amino terminally truncated form of the HTLV env protein which is soluble and accumulates in the cytoplasm of the host cell, so that the HTLV env protein is readily purified from lysed host cells.

The invention is based, in part, on the Applicant's discovery that an antigenic or immunogenic protein having the amino acid sequence of the HTLV-I or the HTLV-II envelope protein with the amino terminal leader or signal sequence deleted, serves to protect the recipient when challenged with an inoculation with HTLV-I or HTLV-II. Furthermore, the transcription of a corresponding cDNA transcript to this novel antigenic protein, in either eucaryotic or procaryotic expression systems, has led to an unexpected abundance of transcribed protein.

The present invention relates to nucleotide sequences that encode the novel antigenic protein, mutants and derivatives thereof. The present invention further relates to methods of expressing the novel antigen, including expression vectors and cell lines, both eucaryotic and procaryotic.

In a preferred embodiment of the invention, the nucleotide sequences, upon expression in an appropriate host cell, produce a polypeptide that is antigenic or immunogenic. Antigenic polypeptides are capable of being immunospecifically bound by an antibody to the antigen. Immunogenic polypeptides are capable of eliciting an immune response to the antigen, e.g., when immunization with the polypeptide elicits production of an antibody that immunospecifically binds the antigen or elicits a cell-mediated immune response directed against the antigen.

In another preferred embodiment of the invention, the antigen protein of the present invention is expressed in a baculovirus system to produce an unglycosylated antigen or the antigen expressed in a stably transfected T cell line to produce a glycosylated antigen. In yet another preferred embodiment of the invention the amino terminally truncated env protein is expressed as a fusion protein to facilitate purification of the protein.

In another aspect of the invention, the method of using these novel antigenic proteins are described. These methods include using these antigenic proteins in vaccine preparations in a solely preventative way, and/or in a therapeutic procedure after the recipient is already infected with either HTLV-I or HTLV-II, or both. The novel antigenic proteins of the invention also have utility in diagnostic immunoassays, passive immunotherapy, and generation of antiidiotypic antibodies.

5.1. NOVEL ENV-GLYCOPROTEIN ANTIGEN

The present invention is based, in part, on the Applicant's surprising discovery that the difficulty in expressing useful amounts of HTLV-env protein could be overcome by expressing an amino truncated form of the HTLV env protein in insect cells or mammalian T lymphocytes. The transcription of a cDNA corresponding to an amino truncated form of the HTLV env protein led to an unexpected abundance of transcribed protein. It was found that an approximate 50 fold increase in expressing of immunologically useful HTLV env protein could be achieved.

The invention is further based on the Applicant's discovery that the antigenicity of this protein is unexpectedly strong. An antigenic protein having the amino acid sequence of the HTLV env protein with the amino terminal leader sequence deleted, induced the production of anti-HTLV-II antibodies in recipients and served to protect the recipient when challenged with an inoculation of HTLV-I or -II. Due to the high level of amino acid sequence identity

between the amino acid sequences of the different regional strains of HTLV-I and HTLV-II, the HTLV-I env antigen of the present invention will serve to protect against the many regional isolates of HTLV-I and the HTLV-II env antigen of the present invention will serve to protect against the many regional isolates of HTLV-II.

In a preferred embodiment of the invention, the antigen protein of the present invention is expressed in a baculovirus system to produce an unglycosylated antigen or the antigen protein is expressed in a stably transfected T cell line to produce a glycosylated antigen.

5.2. NUCLEOTIDE SEQUENCES ENCODING THE ANTIGEN

The present invention encompasses nucleotide sequences encoding the HTLV env protein, including fragments, truncations and variants thereof. A preferred embodiment of the invention encompasses the nucleotide sequences encoding an amino truncated form of the HTLV env gene. The preferred embodiment of the invention encompasses the nucleotide sequences encoding a 33 amino acid truncation of the amino terminal of the HTLV-I env protein. The invention further encompasses nucleotide sequences encoding 1 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, 45 to 50, 50 to 55, 55 to 60, 60 to 65, or 65 to 70 amino acid trunctations of the amino terminal of the HTLV-I env protein. The present invention further encompasses a nucleotide sequence encoding an amino terminally truncated form of the HTLV I env protein such that the polypeptide when expressed recombinantly is not anchored in the membrane of the host cell, yet still retains antigenic activity similar to the full length HTLV I env protein. Further the invention encompasses internal deletions which comprise deleting a sufficient portion of the signal sequence domain so that the polypeptide when expressed recominantly is not anchored in the membrane of the host cell, yet still retains antigenic activity similar to the full length HTLV I env protein. The HTLV-I env nucleotide sequences of the invention include the

following DNA sequences: (1) any DNA sequence encoding a HTLV-I env protein which is immunologically reactive with a anti-HTLV-I env antibody; (2) any DNA sequence encoding a HTLV-I env protein containing the amino acid as shown in FIG. 1; (3) any nucleotide sequence that hybridizes to the complement of the DNA sequence as shown in FIG. 1 under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1mM EDTA at 65°C, and washing in 0.1 x SSC/0.1% SDS at 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology , Vol.I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2·10·3) and encodes a functionally equivalent gene product; (4) any nucleotide sequence that hybridizes to the complement of the DNA sequence as shown in FIG. 1 under less stringent conditions, such as moderately stringent conditions, e.g., washing in 0.2 x SSC/0.1% SDS at 42°C (Ausubel et al., 1989, supra), yet which still encodes a functionally equivalent HTLV env gene product; and (5) any nucleotide sequence that hybridizes to the complement of the DNA sequence as shown in FIG. 1 under less stringent conditions, such as low stringency conditions, e.g., washing in 0.2 x SSC/ 0.1% SDS at 37°C, and encodes a functionally equivalent gene product. A functionally equivalent gene product encompasses a gene product that is produced at high levels and is immunologically reactive with an anti-HTLV-I env antibody.

Another preferred embodiment of the invention encompasses the nucleotide sequences encoding a 17 amino acid truncation of the amino terminal of the HTLV-II env protein. The invention further encompasses nucleotide sequences encoding 1 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 35, 35 to 40, 40 to 45, 45 to 50, 50 to 55, 55 to 60, 60 to 65, or 65 to 70 amino acid truncations of the amino terminal of the HTLV-II env protein. The present invention further encompasses a nucleotide sequence encoding an amino terminally truncated form of the HTLV II env protein such that the polypeptide when expressed recombinantly is not

anchored in the membrane of the host cell, yet still retains antigenic activity similar to the full length HTLV II env protein. Further the invention encompasses internal deletions which comprise deleting a sufficient portion of the signal sequence domain so that the polypeptide when expressed recominantly is not anchored in the membrane of the host cell, yet still retains antigenic activity similar to the full length HTLV II env protein. Further the invention encompasses internal deletions which delete a sufficient portion of the signal sequence domain so that the polypeptide when expressed recombinantly is not anchored. The HTLV-II env nucleotide sequences of the invention include the following DNA sequences: (1) any DNA sequence encoding a HTLV-II env protein which is immunologically reactive with a HTLV-II env antibody; (2) any DNA sequence encoding a HTLV-II env protein containing the amino acid as shown in FIG. 2; (3) any nucleotide sequence that hybridizes to the complement of the DNA sequence as shown in FIG. 2 under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1mM EDTA at 65°C, and washing in 0.1 x SSC/0.1% SDS at 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York, at p. 2103) and encodes a functionally equivalent gene product; (4) any nucleotide sequence that hybridizes to the complement of the DNA sequence as shown in FIG. 2 under less stringent conditions, such as moderately stringent conditions, e.g., washing in 0.2 x SSC/0.1% SDS at 42°C (Ausubel et al., 1989, supra), yet which still encodes a functionally equivalent HTLV-II env gene product; and (5) any nucleotide sequence that hybridizes to the complement of the nucleotide sequence as shown in FIG. 2 under less stringent conditions, such as low stringency conditions, e.g., washing in 0.2 x SSC/0.1% SDS at 37°C, and encodes a functionally equivalent gene product. A functionally equivalent gene product encompasses a gene product that is produced at high

levels and is immunologically reactive with an anti-HTLV-II env antibody.

The present invention also encompasses the expression of nucleotide sequences encoding immunologically equivalent fragments of the HTLV env protein. Such immunologically equivalent fragments of HTLV env may be identified by making analogs of the nucleotide sequence encoding the protein that are truncated at the 5' and/or 3' ends of the sequence and/or have one or more internal deletions, expressing the analog nucleotide sequences, and determining whether the resulting fragments immunologically interact with a HTLV antibody or induce the production of such antibodies in vivo, particularly neutralizing antibodies. For example, a preferred embodiment of the invention encompasses the expression of nucleotide sequences encoding a HTLV env protein with deletions of the amino terminal signal sequence domain and internal regions which may facilitate secretion of the env protein.

The invention also encompasses the DNA expression vectors that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs expression of the coding sequences and genetically engineered host cells that contain any of the foregoing coding sequences operatively associated with a regulatory element that directs the expression of the coding sequences in the host cell. As used herein, regulatory elements include but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements known to those skilled in the art that drive and regulate expression.

The env glycoprotein gene products or peptide fragments thereof, may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the env glycoprotein gene polypeptides and peptides of the invention by expressing nucleic acid containing env glycoprotein gene sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing env glycoprotein

gene product coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, supra, and Ausubel et al., 1989, supra. Alternatively, RNA capable of encoding env glycoprotein gene product sequences may be chemically synthesized using, for example, synthesizers. See, for example, the techniques described in "Oligonucleotide Synthesis", 1984, Gait, M.J. ed., IRL Press, Oxford, which is incorporated by reference herein in its entirety.

The invention also encompasses nucleotide sequences that encode peptide fragments of the HTLV env gene products. In a preferred embodiment of the present invention relates to polypeptides or peptides corresponding to the amino terminally truncated form of the HTLV env protein which is soluble and accumulates in the cytoplasm of the host cell, so that the HTLV env protein is readily purified from lysed host cells. For example, polypeptides or peptides corresponding to the extracellular domain of HTLV env protein may be useful as "soluble" protein which would facilitate secretion. The HTLV env protein gene product or peptide fragments thereof, can be linked to a heterologous epitope that is recognized by a commercially available antibody is also included in the invention. A durable fusion protein may also be engineered; i.e., a fusion protein which has a cleavage site located between the HTLV env sequence and the heterologous protein sequence, so that the HTLV env can be cleaved away from the heterologous moiety. For example, a collagenase cleavage recognition consensus sequence may be engineered between the HTLV env protein or peptide and the heterologous peptide or protein. The HTLV env domain can be released from this fusion protein by treatment with collagenase. In a preferred embodiment of the invention, a fusion protein of glutathione-S-transferase and HTLV env 46 kd protein may be engineered.

5.3. HTLV ENV ANTIGENIC PROTEINS AND POLYPEPTIDES

The present invention relates to a HTLV env polypeptide lacking all or a portion of its signal sequence or membrane spanning domain such that the polypeptide when expressed recombinantly is not anchored in the membrane of the host cell. In a preferred embodiment of the invention, the soluble HTLV env protein is lacking all or a portion of its amino terminus. The preferred embodiment of the invention encompasses the HTLV-I env polypeptide lacking 33 amino acids of the amino terminus and the HTLV-II env polypeptide lacking 17 amino acids of the amino terminus.

The HTLV env protein, polypeptides and peptides, mutated, truncated or deleted forms of the HTLV env proteins can be prepared for vaccine preparations and as pharmaceutical reagents useful in the treatment and prevention of HTLV-I and -II infection.

The env protein has been deduced by its nucleotide sequence to have a hydrophobic signal sequence at its amino terminus, five potential acceptor sites for N-glycosylation linked carbohydrates in the central portion, and a second cluster of hydrophobic amino acids in the putative transmembrane domain (Seiki et al., 1983 Proc. Natl. Acad. Sci. 80:3618-3622). Its sequence character suggests that it has a typical structure of a cell membrane glycoprotein.

The env gene encodes a precursor protein gp63 which undergoes proteolytic cleavage, and subsequent glycosylation to produce two glycoproteins, gp46 and gp21. The gp46 protein constitutes the surface projections observed by electron microscopy on native virus particles, is believed to have receptor binding activity, and contains domains responsible for the production of neutralizing antibodies. The gp21 protein is the transmembrane glycoprotein, and may be involved in cell fusion activity.

In addition, the invention also encompasses proteins that are functionally equivalent to the HTLV env proteins encoded by the nucleotide sequences described in Section

5.2., as judged by a number of criteria, including but not limited to, the ability to be recognized by a HTLV env antibody. Such equivalent HTLV env gene products may contain deletions, additions, substitutions of amino acid residues within the amino acid sequence encoded by the HTLV env gene sequences described above, but which result in a silent change, thus producing a functionally equivalent HTLV env gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine and histidine; and negatively charged amino acids include aspartic acid and glutamic acid. "Functionally equivalent", as utilized herein, refers to a protein capable of being recognized by a HTLV env antibody, that is a protein capable of eliciting a substantially similar immunological response as the endogenous HTLV env gene products described above.

While random mutations can be made to the HTLV env nucleotide sequences (using random mutagenesis techniques well known to those skilled in the art) and the resulting HTLV env proteins tested for activity, site directed mutations of the HTLV env coding sequence can be engineered (using site-directed mutagenesis techniques well known to those in the art) to generate mutant HTLV env proteins with increased function, e.g., leading to enhanced expression or antigenicity.

The HTLV env proteins of the present invention for use in vaccine preparations are substantially pure or homogenous. The protein is considered substantially pure or homogenous when at least 60 to 75% of the sample exhibits a single polypeptide sequence. A substantially pure protein will preferably comprise 60 to 90% of a protein sample, more

preferably about 95% and most preferably 99%. Methods which are well known to those skilled in the art can be used to determine protein purity or homogeneity, such as polyacrylamide gel electrophoresis of a sample, followed by visualizing a single polypeptide band on a staining gel. Higher resolution may be determined using HPLC or other similar methods well known in the art.

The present invention encompasses polypeptides which are typically purified from host cells expressing recombinant nucleotide sequences encoding these proteins. Such protein purification can be accomplished by a variety of methods well known in the art. In a preferred embodiment, the HTLV env protein of the present invention is expressed as a fusion protein with glutathione-S-transferase. The resulting recombinant fusion proteins purified by affinity chromatography and the HTLV env domain is cleaved away from the heterologous moiety resulting in a substantially pure HTLV env protein sample. Other methods may be used, see for example, the techniques described in "Methods In Enzymology", 1990, Academic Press, Inc., San Diego, "Protein Purification: Principles and practice", 1982, Springer-Verlag, New York.

5.4. EXPRESSION SYSTEMS

The present invention encompasses expression systems, both eucaryotic and procaryotic expression vectors, which may be used to express both truncated and full-length forms of the HTLV env protein.

In a preferred embodiment of the invention, the nucleotide sequences of FIG. 1, deleted of the boxed region, encoding the truncated HTLV-I env protein are expressed in either eucaryotic or procaryotic expression vectors. In another preferred embodiment of the invention, the nucleotide sequences of FIG. 2, deleted of the boxed region, encoding the truncated HTLV-II env protein are expressed in either eucaryotic or procaryotic expression vectors.

A preferred embodiment of the invention encompasses the expression of both full-length and truncated forms of the

HTLV env gene products in a baculovirus system to produce an unglycosylated antigen.

Another preferred embodiment of the invention encompasses the expression of full-length and truncated forms of the HTLV env gene products in a stably transfected T cell line to produce a glycosylated antigen.

A variety of host-expression vector systems may be utilized to express the env glycoprotein gene coding sequences of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, exhibit the env glycoprotein gene product of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing env glycoprotein gene product coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing the env glycoprotein gene product coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the env glycoprotein gene product coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing env glycoprotein gene product coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the env glycoprotein gene product being expressed. For

example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of env glycoprotein protein or for raising antibodies to env glycoprotein protein, for example, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the env glycoprotein gene product coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic Acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The env glycoprotein gene coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of env glycoprotein gene coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed. (E.g., see Smith et al., 1983, J. Virol. 46: 584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the env glycoprotein gene coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing env glycoprotein gene product in infected hosts. (E.g., See Logan & Shenk, 1984, Proc. Natl. Acad. Sci. USA 81:3655-3659). Specific initiation signals may also be required for efficient translation of inserted env glycoprotein gene product coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire env glycoprotein gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the env glycoprotein gene coding sequence is inserted, exogenous translational control signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:516-544).

5.5. CELL LINES

The present invention encompasses the expression of HTLV env glycoprotein in animal and insect cell lines. In a preferred embodiment of the present invention, the env glycoprotein is expressed in a baculovirus vector in an insect cell line to produce an unglycosylated antigen. In another preferred embodiment of the invention, the env glycoprotein is expressed in a stably transfected T lymphocyte cell line to produce a glycosylated antigen.

Host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g. cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification of the foreign protein expressed. To this end, eucaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3 and WI38 cell lines.

For long term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the env glycoprotein gene product may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g.,promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant

plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines. This method may advantageously be used to engineer cell lines which express the env glycoprotein gene products. Such cell lines would be particularly useful in screening and evaluation of compounds that affect the endogenous activity of the env glycoprotein gene product.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA 77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, Proc. Natl. Acad. Sci. USA 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and hygro, which confers resistance to hygromycin (Santerre, et al., 1984, Gene 30:147).

Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht, et al., 1991, Proc. Natl. Acad. Sci. USA 88: 8972-8976). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus

are loaded onto Ni^{2+} nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

5.6. VACCINE FORMULATIONS AND METHODS OF ADMINISTRATION

Since the HTLV env protein antigen of the present invention can be produced in large amounts, the antigen thus produced and purified has use in vaccine preparations. The HTLV env protein also has utility in immunoassays, e.g., to detect or measure in a sample of body fluid from a vaccinated subject the presence of antibodies to the antigen, and thus to diagnose infection and/or to monitor immune response of the subject subsequent to vaccination.

The preparation of vaccines containing an immunogenic polypeptide as the active ingredient is known to one skilled in the art.

5.6.1. DETERMINATION OF VACCINE EFFICACY

The immunopotency of the HTLV env antigen can be determined by monitoring the immune response in test animals following immunization with the HTLV env antigen, or by use of any immunoassay known in the art. Generation of a humoral (antibody) response and/or cell-mediated immunity, may be taken as an indication of an immune response. Test animals may include mice, hamsters, dogs, cats, monkeys, rabbits, chimpanzees, etc., and eventually human subjects.

Methods of introducing the vaccine may include oral, intracerebral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal or any other standard routes of immunization. The immune response of the test subjects can be analyzed by various approaches such as: the reactivity of the resultant immune serum to the HTLV env antigen, as assayed by known techniques, e.g., immunosorbant assay (ELISA), immunoblots, radioimmunoprecipitations, etc., or in the case where the HTLV env antigen displays antigenicity or immunogenicity, by protection of the

immunized host from infection by HTLV and/or attenuation of symptoms due to infection by HTLV in the immunized host.

As one example of suitable animal testing of an HTLV env vaccine, the vaccine of the invention may be tested in rabbits for the ability to induce an antibody response to the HTLV env antigen. Male specific-pathogen-free (SPF) young adult New Zealand White rabbits may be used. The test group each receives a fixed concentration of the vaccine. A control group receives an injection of 1 mM Tris-HCl pH 9.0 without the HTLV env antigen.

Blood samples may be drawn from the rabbits every one or two weeks, and serum analyzed for antibodies to the HTLV env protein. The presence of antibodies specific for the antigen may be assayed, e.g., using an ELISA.

5.6.2. VACCINE FORMULATIONS

Suitable preparations of such vaccines include injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, suspension in, liquid prior to injection, may also be prepared. The preparation may also be emulsified, or the polypeptides encapsulated in liposomes. the active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine preparation may also include minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine.

Examples of adjuvants which may be effective, include, but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine.

The effectiveness of an adjuvant may be determined by measuring the induction of antibodies directed against an immunogenic polypeptide containing a HTLV env polypeptide epitope, the antibodies resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

The polypeptides may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids, such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with free carboxyl groups may also be derived from inorganic bases, such as, for example, sodium potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

The vaccines of the invention may be multivalent or univalent. Multivalent vaccines are made from recombinant viruses that direct the expression of more than one antigen.

Many methods may be used to introduce the vaccine formulations of the invention; these include but are not limited to oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal routes, and via scarification (scratching through the top layers of skin, *e.g.*, using a bifurcated needle).

The patient to which the vaccine is administered is preferably a mammal, most preferably a human, but can also be a non-human animal including but not limited to cows, horses, sheep, pigs, fowl (*e.g.*, chickens), goats, cats, dogs, hamsters, mice and rats.

The vaccine formulations of the invention comprise an effective immunizing amount of the HTLV env protein and a pharmaceutically acceptable carrier or excipient. Vaccine preparations comprise an effective immunizing amount of one or more antigens and a pharmaceutically acceptable carrier or

excipient. Pharmaceutically acceptable carriers are well known in the art and include but are not limited to saline, buffered saline, dextrose, water, glycerol, sterile isotonic aqueous buffer, and combinations thereof. One example of such an acceptable carrier is a physiologically balanced culture medium containing one or more stabilizing agents such as stabilized, hydrolyzed proteins, lactose, etc. The carrier is preferably sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile diluent can be provided so that the ingredients may be mixed prior to administration.

In a specific embodiment, a lyophilized HTLV env polypeptide of the invention is provided in a first container; a second container comprises diluent consisting of an aqueous solution of 50% glycerin, 0.25% phenol, and an antiseptic (*e.g.*, 0.005% brilliant green).

The precise dose of vaccine preparation to be employed in the formulation will also depend on the route of administration, and the nature of the patient, and should be decided according to the judgment of the practitioner and each patient's circumstances according to standard clinical techniques. An effective immunizing amount is that amount

sufficient to produce an immune response to the antigen in the host to which the vaccine preparation is administered.

Use of purified antigens as vaccine preparations can be carried out by standard methods. For example, the purified protein(s) should be adjusted to an appropriate concentration, formulated with any suitable vaccine adjuvant and packaged for use. Suitable adjuvants may include, but are not limited to: mineral gels, *e.g.*, aluminum hydroxide; surface active substances such as lysolecithin, pluronic polyols; polyanions; peptides; oil emulsions; alum, and MDP. The immunogen may also be incorporated into liposomes, or conjugated to polysaccharides and/or other polymers for use in a vaccine formulation. In instances where the recombinant antigen is a hapten, *i.e.*, a molecule that is antigenic in that it can react selectively with cognate antibodies, but not immunogenic in that it cannot elicit an immune response, the hapten may be covalently bound to a carrier or immunogenic molecule; for instance, a large protein such as serum albumin will confer immunogenicity to the hapten coupled to it. The hapten-carrier may be formulated for use as a vaccine.

Effective doses (immunizing amounts) of the vaccines of the invention may also be extrapolated from dose-response curves derived from animal model test systems.

The invention also provides a pharmaceutical pack or kit comprising one or more containers comprising one or more of the ingredients of the vaccine formulations of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

The present invention thus provides a method of immunizing an animal, or treating or preventing various diseases or disorders in an animal, comprising administering to the animal an effective immunizing dose of a vaccine of the present invention.

5.6.3. USE OF ANTIBODIES GENERATED BY THE VACCINES OF THE INVENTION

The antibodies generated against the antigen by immunization with the HTLV env protein of the present invention also have potential uses in diagnostic immunoassays, passive immunotherapy, and generation of antiidiotypic antibodies.

The generated antibodies may be isolated by standard techniques known in the art (*e.g.*, immunoaffinity chromatography, centrifugation, precipitation, etc.) and used in diagnostic immunoassays. The antibodies may also be used to monitor treatment and/or disease progression. Any immunoassay system known in the art, such as those listed *supra*, may be used for this purpose including but not limited to competitive and noncompetitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme-linked immunosorbent assays), "sandwich" immunoassays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays and immunoelectrophoresis assays, to name but a few.

The vaccine formulations of the present invention can also be used to produce antibodies for use in passive immunotherapy, in which short-term protection of a host is achieved by the administration of pre-formed antibody directed against a heterologous organism.

The antibodies generated by the vaccine formulations of the present invention can also be used in the production of antiidiotypic antibody. The antiidiotypic antibody can then in turn be used for immunization, in order to produce a subpopulation of antibodies that bind the initial antigen of the pathogenic microorganism (Jerne, 1974, Ann. Immunol. (Paris) 125c:373; Jerne, et al., 1982, EMBO J. 1:234).

In immunization procedures, the amount of immunogen to be used and the immunization schedule will be determined by a physician skilled in the art and will be administered by

reference to the immune response and antibody titers of the subject.

5.6.4. PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labelled for treatment of an indicated condition.

6. EXAMPLE: EXPRESSION OF HTLV-II ENV PROTEINS

In the example presented herein, the expression of an amino terminally truncated form of the HTLV env protein is demonstrated. It is further demonstrated that the resulting recombinant HTLV env protein is immunoreactive.

6.1. MATERIALS AND METHODS

Construction of baculovirus transfer vector and GST fusion vector.

The nucleotide sequence of the HTLV-II env gene used in these constructs is shown in FIG. 2. The nucleotide sequences deleted from the HTLV-II env gene are indicated by the boxed region in FIG. 2. The primers used to amplify the truncated form of HTLV-II env gene are underlined in FIG. 2.

The nucleotide sequence of the HTLV-I env gene that may be expressed in the baculotransfer vector and GST fusion vector is shown in FIG. 1. The nucleotide sequences deleted from the HTLV-I env gene are indicated by the boxed region in FIG. 1. The primers used to amplify the truncated form of the HTLV-I env gene are underlined in FIG. 1.

HTLV-II env gene was amplified by PCR from plasmid Mo which contains the 3′ half genome (Shimotono) using taq

polymerase (Cetus), 30 cycles of amplification (94°C, 30 sec-56 °C, 30 sec,'72°C, 1 min) and the following oligonucleotide primers: 5'-AAGGATCCATGGGTAATGTTTTCTTC-3'5180-5197 and 5'-AAGGATCCTTATAGCATGGTTTCTGG-3'6643-6626 (BamHl site is indicated as underlined and sequence numbers were derived from the published sequence of HTLV-II-Mo (Shimotono). PCR-amplified products was digested with BamHl and following electrophoresis, purified in low-melting temperature agarose gels; DNA bands were excised and ligated to baculovirus transfer vector pVL1 392 at the BamH I site which is located downstream of the polyhedron promotor. The baculovirus vector used to make these constructs is pVL1 392 was obtained from Invitrogen, San Diego, CA. This recombinant plasmid was used to transfect insect cells.

Similarly fusion proteins of glutathione S-transferase (GST) and HTLV-11 env cleaved protein, gp46 was prepared by amplifying plasmid Mo-T with primers 5'-AAGGATCCATGGGTAATGTTTTCTTC3'(5180-5197) and 5'AAGAATTCACGGCGGCGTCTTGTCGCGCCAGG3'(6103-6086, BamHI and EcoRI stickey ends were introduced with these primers and used to ligate the fragments together. Using the expression plasmid pGEX2T (Pharmacia, Upsala, Sweden) GST-gp46 protein was expressed and purified according to the manufacturer's instructions.

Production of recombinant baculovirus

To generate recombinant baculovirus, monolayers consisting 10^6 insect cells (High Five(H5), Invitrogen, San Diego, CA) were cotransfected with transfer vector DNA containing env CDNA as described above, together with linearized baculovirus DNA (Baculogold, PharMingen, San Diego, CA), using calciumphosphate method. Single plaque including recombinant virus was purified from supernatant and amplified in H5 monolayers cells.

Western immunoblot analysis

Western blot was used to assess the reactivity of the env protein expressed in baculovirus infected cells with human antisera. Total cell extracts from H5 cells infected with recombinant baculovirus were subject to electrophoresis through 10% SDS polyacrylamide gels (SDS-PAGE) and transferred to PVDF membrane (Immobilon, Bedford, MA). Filters were probed with HTLV-11 infected patient's serum (Hall) at 200 times dilutions. Bound antibody was detected by inoculation of the filter with horseradish'peroxidase-conjugated antibody, anti-human (DAKO A/S, Denmark), at a 1:5000 dilution, followed by development with chemiluminescence (ECL, Amerciam, Buckinghamshire, England)

Cells and viruses

The T-cell lines CEM was used for T cell control negative for HTLV-II, and B-cell line, BJAB for fusion assay. HTLV-II-Vines was isolated from a male intravenous drug abuser who was not infected with human immunodeficiency virus and was used to establish an HTLV-II carrying human lymphoid cell line (Hall). HTLV-II-Mo-T is a HTLV-II-infected lymphoblastoid T cell line from patient Mo with a T-cell variant of hairy cell leukemia (Saladoon). All the cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 2%glutamine and 50 ug/ml of gentamycine, and cultured at 37°C in 5% CO_2.

The insect cell, High Five (H5, Invitrogen, San Diego, CA) was maintained in TC100 medium (Gibco BRL, Gaithersburg, MD) including 10% of calf fetal serum and 50ug/ml of kanamycin.

6.2. RESULTS

The purified recombinant baculovirus containing HTLV-II env gene was used to infect monolayers of H5 insect cells. The infected insect cells were harvested 4 days after infection and examined for expression of the HTLV-II envelope polypeptides. Proteins from lysed cells were separated by polyacrylamide gel and transferred to PVDF membrane and

probed with HTLV-II infected patient's sera(Hall) (FIG. 2). A protein with an apparent molecular mass of 63 kDa was immunoreactive.

The location of the recombinant protein gp63 (rgp63) in the insect cells was examined by immunofluorescence. Similarly infected insect cells were harvested 2 days after infection and incubated HTLV-II infected patient's serum. The majority of infected insect cells bound the antibody, suggesting recombinant gp63 localizing to the surface of infected insect cells (data not shown).

These results demonstrate that an immunoreactive HTLV env antigen was successfully expressed in a baculovirus infected insect cell line.

7. EXAMPLE: IMMUNIZATION WITH HTLV-II ENV PROTEINS

The following analysis was conducted to determine the effects of inoculating rabbits with the recombinant gp63 env protein. In this analysis, rabbits were immunized with gp63 expressing insect cells, and serum from the rabbits was assayed for antibodies to the HTLV env protein. The presence of anti-HTLV env antibodies was measured by: (1) the ability to detect recombinant GST-gp46 fusion protein expressed in bacteria, and (2) the ability to recognize HTLV-II infected human cells in FACS analysis.

7.1. MATERIALS AND METHODS

Cells and viruses

The T-cell lines CEM was used for T cell control negative for HTLV-II, and B-cell line, BJAB for fusion assay. HTLV-II-Vines was isolated from a male intravenous drug abuser who was not infected with human immunodeficiency virus and was used to establish an HTLV-II carrying human lymphoid cell line(Hall). HTLV-II-Mo-T is a HTLV-II-infected lymphoblastoid T cell line from patient Mo with a T-cell variant of hairy cell leukemia(Saladoon). All the cell lines were maintained in RPMI 1640 medium supplemented with 10%

fetal calf serum, 2% glutamine and 50 ug/ml of gentamycine, and cultured at 37°C in 5% CO_2.

The insect cell, High Five (H5, Invitrogen, San Diego, CA) was maintained in TC1 00 medium (Gibco BRL, Gaithersburg, MD) including 10% of calf fetal serum and 50ug/ml of kanamycin.

FACS analysis

HTLV-II-Vines, Mo-T, and CEM cell lines were stained with immunized rabbits sera, following 3 times of washing with PBS incubated with FITC conjugated goat anti-human sera (DAKO A/S, Denmark) at 1-50, in the presence of 2% calf fetal serum. Relative fluorescence intensity was detected by flow cytometry.

Rabbits

2.5kg, specific pathogen free, female New Zealand White rabbits were obtained from a commercial rabbitry (SCL, Shizuoka, Japan). Groups of rabbits were inoculated intravenously with $5x10^7$ HTLV-II infected cells or heat inactivated cells (70°C for 20 min.) as shown Table 1. HTLV-II infected cells were 90% infected, as determined by fluorescent antibody assay using an HTLV-II infected patient's serum (FIG. 5).

Syncytium inhibition assay.

HTLV-II-Vines cells were suspended in RPMI medium at 10^6 cells per ml, aliquots (50ul per well) were incubated with heat-inactivated, 100ul of diluted Rabbit serum in 96-well plates at 37°C for 15 min, and then 50ul of BJAB cell suspension(10^6 cells per ml) was added to each well. After incubation at 37°C for 16 hrs in a 5% CO_2 incubator, each well was examined for syncytia (giant multinuclear cells) with an inverted microscope. Neutralization titers of antibody samples were expressed as the reciprocal of the sample dilution at which the syncytium formation was completely (100%) inhibited in the microcultures.

Immunization of Rabbits.

Recombinant-baculovirus-infected H5 cells were pelleted, washed once in PBS, and resuspended in PBS at a concentration of 10^7 cells/ml. Samples (10^7 cells) which were emulsified in complete (day 0) or incomplete (day 14, 28, and 42) Freund's adjuvant, were injected intramuscularly into New Zealand White female rabbits (2.5kg). Rabbits were purchased from SLC, Shizuoka, Japan. Immune sera from rabbits were collected on day 56.

7.2. RESULTS

Rabbits were immunized with recombinant gp63 expressing insect cells, and the serum was assayed for the detection of recombinant GST-fusion protein with a cleaved form of the env protein, gp46, expressed in bacteria. Because antigenicity of insect cells are different from bacteria, rabbit antisera after immunization does not show cross reactivity to this fusion protein. One week after immunization, serum from Rabbit 3 and 4 showed reactivity against GST-gp46 (FIG. 4, lane 2 and 3), however the serum of R3 prior to immunization did not react (lane 1).

Rabbits immunized with recombinant gp63 expressing insect cells, and the serum was assayed for the detection of HTLV-II infected human cells by FACS analysis. The FACS analysis showed positive staining in the cell lines, Vines and Mo-T (FIG. 5). In contrast the uninfected T cell line, CEM showed a negative staining pattern, suggesting that the rabbit serum is including antibody against HTLV-II env proteins.

TABLE 1

	IMMUNIZATION	CHALLENGE OF HTLV-II-Vines	DETECTION OF PROVIRUS (weeks post infection) 2	4	6	8	20	70
Exp 1								
R1	-	5×10^7	-	+	+	+	+	+
R2	-	5×10^7	+	+	+	+	+	+
R3	+	5×10^7						
R4	+	5×10^7	—	—	—	—	—	—
			—	—	—	—	—	—
Exp 2								
R5	-	5×10^7					+	+
R6	-	5×10^7 (heat inactivat)					-	-
R7	-	5×10^7 (heat inactivat)					-	-
R8	-	5×10^7					-	-
R9	+	5×10^7					-	-

8. NEUTRALIZATION ACTIVITY OF SERA FROM VACCINATED ANIMALS

HTLV-II infected cells induce cell to cell fusion after cocultivation with B-cell line, BJAB (Hall). Therefore, serum from rabbits immunized with recombinant gp63 was assayed for its ability to block cell to cell fusion.

8.1. MATERIALS AND METHODS

Rabbits

2.5kg, specific pathogen free, female New Zealand White rabbits were obtained from a commercial rabbitry(SCL, Shizuoka, Japan). Groups of rabbits were inoculated intravenously with 5×10^7 HTLV-II infected cells or heat inactivated cells (70°C for 20 min.) as shown Table 1. HTLV-II infected cells were 90% infected, as determined by fluorescent antibody assay using an HTLV-II infected patient's serum (FIG. 4).

Syncytium inhibition assay.

HTLV-II-Vines cells were suspended in RPMI medium at 10^6 cells per ml, aliquots (50ul per well) were incubated with heat-inactivated, 100ul of diluted Rabbit serum in 96-well plates at 37°C for 15 min, and then 50ul of BJAB cell suspension (10^6 cells per ml) was added to each well. After incubation at 37°C for 16 hrs in a 5% CO_2 incubator, each well was examined for syncytia (giant multinuclear cells) with an inverted microscope. Neutralization titers of antibody samples were expressed as the reciprocal of the sample dilution at which the syncytium formation was completely (100%) inhibited in the microcultures.

Immunization of Rabbits.

Recombinant-baculovirus-infected H5 cells were pelleted, washed once in PBS, and resuspended in PBS at a concentration of 10 7 cells/ml. Samples (10^7 cells) which were emulsified in complete (day 0) or incomplete (day 14, 28, and 42) Freund's adjuvant, were injected intramuscularly into New Zealand White female rabbits (2.5kg). Rabbits were purchased from SLC, Shizuoka, Japan. Immune sera from rabbits were collected on day 56.

Particle Agglutination method

The titer of rabbit sera against HTLV-II antigen was calculated by particle agglutination kit (Fujirebio inc., Tokyo, Japan). Beads attached with purified HTLV-I particles were incubated with various dilutions of sera and maximum dilution that leads to agglutination were described as antibody titers.

8.2. RESULTS

The results of incubating HTLV-II infected cells with various dilutions of antisera to gp62 demonstrated that at most 1:50 dilution of sera from samples R3, R4, R8 and R9 was enough to completely inhibit fusion at the first bleeding. Fusion was blocked by incubation with antisera but not with

preimmune sera. This suggests that the epitopes eliciting the fusion activity are located within the gp63, env protein. Previous studies showed that sera that can block cell fusion always show neutralization of virus infection.

In addition, since a cell free infection is not possible in HTLV-I and II infection, blockage of cell to cell fusion induced by the rabbit gp63 immunized sera indicates blockage of HTLV-II infection. Therefore these results indicate that rabbit gp63 immunized sera has neutralizing activity against HTLV-II.

9. PROTECTION OF RABBITS FROM HTLV-II INFECTION

In the Example presented herein, the ability of the HTLV env antigen of the present invention was assayed for its ability to protect rabbits against HTLV-II infection.

9.1. MATERIALS AND METHODS

HTLV-II challenge and detection of provirus by PCR.

HTLV-II-Vines cells were washed with PBS and injected intravenously into rabbits($5x10^7$ cells) after with heat-inactivation or without inactivation. Every week postinfection, PBL were isolated from heparinized blood samples by density separation medium for rabbit lymphocytes, lympholyte-Rabbit (Cedarlane Laboratories, Hornby, Ontario, Canada).

DNA samples were prepared by DNAzol (Gibco BRL, Gaitherburg, MD) from PBMC, and 1 ug DNA samples were subjected to PCR analysis. The primers used for PCR to amplify tax region were SK43 5'TGGATA CCC CGT CTA CGT GT3' (7248 to 7267) and SK44, 5'GAG CTG ACA ACG CGT CCA TCG3' (7406 to 7386), and those used for the second step of PCR were SK43', 5GCG ATT GTG TAC AGG CCG ATT GGT3'(7271 to 7294) which locates just downstream of SK43 and together with SK44 works as nested primer.

(SK43 and 44 were derived from PCR protocols M.A. Innis, David H. Gelfand, J.J. Sninsky and T.J. White. Academic press.)

Southern hybridization

Ten microliters (from a 50-ul PCR samples) of nested amplified DNA was separated on 1.5% agarose gels and blotted to nylon membranes (Schlicher & Schuell). Membranes were hybridized for 2 hrs at 68°C in HybriQuick solution (Strategen, San Diego, CA). PCR fragment made with SK43 and 44 primers, were purified from agarose gel and was used for probe after random-primed-labelled with (α-^{32}P)dCTP.

9.2. RESULTS

Rabbits were inoculated intravenously with $5x10^7$ HTLV-II-Vines cells. In the first experiment, the nonimmunized groups R1 and R2 were seroconverted for HTLV-II 2 weeks after challenge, with the antibody titer rising to a maximum at the following 8 weeks (FIG. 4). Western blot analysis of R1 demonstrated the presence of antibody against the recombinant cleaved env-fusion-protein after challenge (FIG. 6). In rabbits, R3 and R4, immunized with HTLV-II env expressing insect cells, respectively, antibody titer continued to plateau in the following 10 weeks after challenge (FIG. 5).

The presence of HTLV-II nucleotide sequences in peripheral blood lymphocytes (PBL) isolated from the immunized rabbits was assayed by PCR analysis. In the first experiment, as summarized in table 1, HTLV-II provirus was detected in DNA samples from PBLs of nonimmunized rabbits but was not been detected in PBLs from immunized rabbits for 20 weeks. Also in the second challenge HTLV-II provirus was detected only in immunized rabbits. To confirm HTLV-II infection, heat inactivated HTLV-II-Vines was also challenged, expectedly provirus was not detected after 20 weeks. To confirm the absence of provirus in immunized rabbits and the presence of provirus in non-immunized rabbits, HTLV tax gene PCR products were amplified after both challenges subjected to southern hybridization (FIG. 6). The results of the second PCR analysis demonstrated that after challenge with HTLV-II, non-immunized rabbits contained HTLV provirus, while in immunized rabbits no HTLV-II provirus

could be detected. These results indicate that the HTLV env antigen of the present invention conferred protection in immunized rabbits against challenge with the HTLV-II virus.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. An antigenic or immunogenic polypeptide having an amino acid sequence corresponding to the HTLV-II envelope protein deleted of a sufficient portion of the leader sequence domain so that the polypeptide when expressed by a genetically engineered host cell is not anchored in the membrane of the host cell, or any analog thereof, and a pharmaceutically acceptable carrier.

2. An antigenic or immunogenic polypeptide having an amino acid sequence corresponding to the HTLV-I envelope protein deleted of a sufficient portion of the leader sequence domain so that the polypeptide when expressed by a genetically engineered host cell is not anchored in the membrane of the host cell, or any analog thereof, and a pharmaceutically acceptable carrier.

3. A method of treating or preventing a disease or disorder in a subject caused by infection with HTLV-II comprising administering to the subject the polypeptide of Claim 1 in an amount sufficient to protect the subject against challenge with the HTLV-II virus.

4. The method of Claim 3, wherein the recombinant protein is produced by a baculovirus insect cell expression system.

5. The method of Claim 3, wherein the amino terminal signal sequence of the recombinant protein is truncated.

6. The method of Claim 3, wherein the recombinant protein is expressed in a T cell line.

7. A method of treating or preventing a disease or disorder in a subject caused by infection with HTLV-I comprising administering to the subject the polypeptide of

Claim 2 in an amount sufficient to protect the subject against challenge with the HTLV-I virus.

8. The method of Claim 7, wherein the recombinant protein is produced by a baculovirus insect cell expression system.

9. The method of Claim 7, wherein the terminal signal sequence of the recombinant protein is truncated.

10. The method of Claim 7, wherein the recombinant protein is expressed in a T cell line.

11. An immunogenic polypeptide having the amino acid sequence of SEQ ID NO.: 3, corresponding to a truncated gp63 subunit of the HTLV-II envelope protein lacking the amino terminal leader sequence, or any analog thereof.

12. An immunogenic polypeptide having the amino acid sequence of SEQ ID NO.: 1, corresponding to a truncated gp63 subunit of the HTLV-I envelope protein lacking the amino terminal leader sequence, or any analog thereof.

13. A method of eliciting in a subject the production of antibodies which specifically bind the HTLV-II envelope protein comprising administering to the subject the polypeptide of Claim 1.

14. A method of eliciting in a subject the production of antibodies which specifically bind the HTLV-I envelope protein comprising administering to the subject the polypeptide of Claim 2.

15. An antigenic or immunogenic polypeptide having the amino acid sequence corresponding to the HTLV-II envelope protein having a deletion of 33 amino acids from the leader sequence domain.

16. An antigenic or immunogenic polypeptide having the amino acid sequence corresponding to the HTLV-I envelope protein having a deletion of 17 amino acids from the leader sequence domain.

17. A method of generating an immune response in a subject treating or preventing a disease in a subject caused by infection with HTLV-II comprising administering to the subject the polypeptide of Claim 15 in an amount sufficient to elicit the production of HTLV-II specific antibodies in the subject.

18. A method of treating or preventing a disease in a subject caused by infection with HTLV-I comprising administering to the subject the antigenic or immunogenic polypeptide of Claim 16 in an amount sufficient to increase an HTLV-I specific immune response in the subject.

19. The method of Claim 17, wherein the amino terminal signal sequence of the recombinant protein is truncated.

20. The method of Claim 17, wherein the recombinant protein is expressed in a T cell line.

21. The method of Claim 17, wherein the recombinant protein is produced by a baculovirus insect cell expression system.

22. The method of Claim 18, wherein the amino terminal signal sequence of the recombinant protein is truncated.

23. The method of Claim 18, wherein the recombinant protein is expressed in a T cell line.

24. The method of Claim 18, wherein the recombinant protein is produced by a baculovirus insect cell expression system.

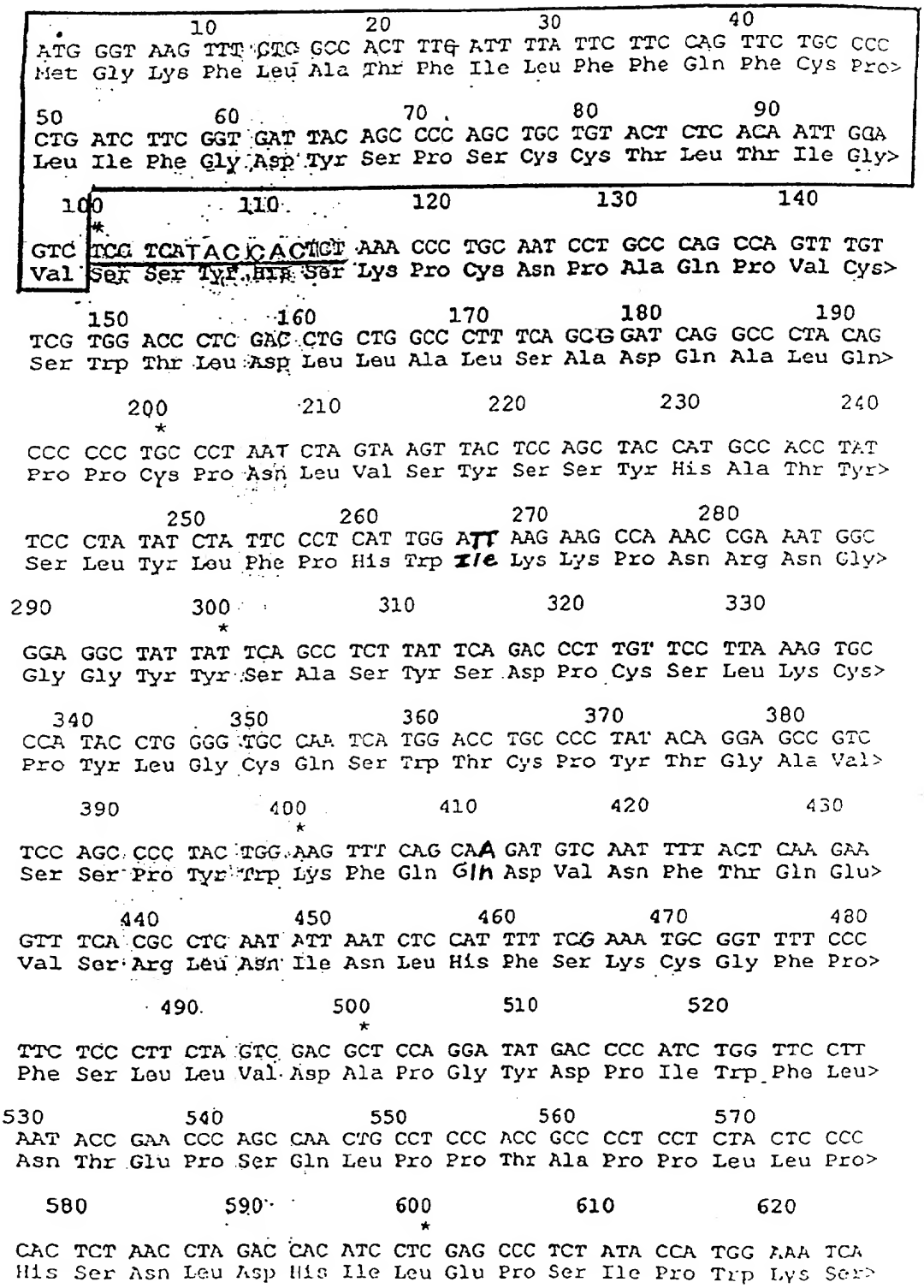
10 20 30 40
ATG GGT AAG TTT CTG GCC ACT TTG ATT TTA TTC TTC CAG TTC TGC CCC
Met Gly Lys Phe Leu Ala Thr Phe Ile Leu Phe Phe Gln Phe Cys Pro>
50 60 70 80 90
CTG ATC TTC GGT GAT TAC AGC CCC AGC TGC TGT ACT CTC ACA ATT GGA
Leu Ile Phe Gly Asp Tyr Ser Pro Ser Cys Cys Thr Leu Thr Ile Gly>
100 110 120 130 140
GTC TCG TCA TAC CAC TCT AAA CCC TGC AAT CCT GCC CAG CCA GTT TGT
Val Ser Ser Tyr His Ser Lys Pro Cys Asn Pro Ala Gln Pro Val Cys>
150 160 170 180 190
TCG TGG ACC CTC GAC CTG CTG GCC CTT TCA GCG GAT CAG GCC CTA CAG
Ser Trp Thr Leu Asp Leu Leu Ala Leu Ser Ala Asp Gln Ala Leu Gln>
200 210 220 230 240
CCC CCC TGC CCT AAT CTA GTA AGT TAC TCC AGC TAC CAT GCC ACC TAT
Pro Pro Cys Pro Asn Leu Val Ser Tyr Ser Ser Tyr His Ala Thr Tyr>
250 260 270 280
TCC CTA TAT CTA TTC CCT CAT TGG ATT AAG AAG CCA AAC CGA AAT GGC
Ser Leu Tyr Leu Phe Pro His Trp Ile Lys Lys Pro Asn Arg Asn Gly>
290 300 310 320 330
GGA GGC TAT TAT TCA GCC TCT TAT TCA GAC CCT TGT TCC TTA AAG TGC
Gly Gly Tyr Tyr Ser Ala Ser Tyr Ser Asp Pro Cys Ser Leu Lys Cys>
340 350 360 370 380
CCA TAC CTG GGG TGC CAA TCA TGG ACC TGC CCC TAT ACA GGA GCC GTC
Pro Tyr Leu Gly Cys Gln Ser Trp Thr Cys Pro Tyr Thr Gly Ala Val>
390 400 410 420 430
TCC AGC CCC TAC TGG AAG TTT CAG CAA GAT GTC AAT TTT ACT CAA GAA
Ser Ser Pro Tyr Trp Lys Phe Gln Gln Asp Val Asn Phe Thr Gln Glu>
440 450 460 470 480
GTT TCA CGC CTC AAT ATT AAT CTC CAT TTT TCG AAA TGC GGT TTT CCC
Val Ser Arg Leu Asn Ile Asn Leu His Phe Ser Lys Cys Gly Phe Pro>
490 500 510 520
TTC TCC CTT CTA GTC GAC GCT CCA GGA TAT GAC CCC ATC TGG TTC CTT
Phe Ser Leu Leu Val Asp Ala Pro Gly Tyr Asp Pro Ile Trp Phe Leu>
530 540 550 560 570
AAT ACC GAA CCC AGC CAA CTG CCT CCC ACC GCC CCT CCT CTA CTC CCC
Asn Thr Glu Pro Ser Gln Leu Pro Pro Thr Ala Pro Pro Leu Leu Pro>
580 590 600 610 620
CAC TCT AAC CTA GAC CAC ATC CTC GAG CCC TCT ATA CCA TGG AAA TCA
His Ser Asn Leu Asp His Ile Leu Glu Pro Ser Ile Pro Trp Lys Ser>

Fig. 1

```
     630          640          650          660          670
AAA CTC CTG ACC CTT GTC CAG TTA ACC CTA CAA AGC ACT AAT TAT ACT
Lys Leu Leu Thr Leu Val Gln Leu Thr Leu Gln Ser Thr Asn Tyr Thr>

        680          690          700          710          720
                                     *
TGC ATT GTC TGT ATC GAT CGT GCC ACC CTA TCC ACT TGG CAC GTC CTA
Cys Ile Val Cys Ile Asp Arg Ala Thr Leu Ser Thr Trp His Val Leu>

          730          740          750          760
TAC TCT CCC AAC GTC TCT GTT CCA TCC TCT TCT TCT ACC CCC CTC CTT
Tyr Ser Pro Asn Val Ser Val Pro Ser Ser Ser Ser Thr Pro Leu Leu>

770          780          790          800          810
                                          *
TAC CCA TCG TTA GCG CTT CCA GCC CCC CAC CTG ACG TTA CCA TTT AAC
Tyr Pro Ser Leu Ala Leu Pro Ala Pro His Leu Thr Leu Pro Phe Asn>

  820          830          840          850          860
TGG ACC CAC TGC TTT GAC CCC CAG ATT CAA GCT ATA GTC TCC TCC CCC
Trp Thr His Cys Phe Asp Pro Gln Ile Gln Ala Ile Val Ser Ser Pro>

    870          880          890          900          910
                                          *
TGT CAT AAC TCC CTC ATC CTG CCC CCC TTT TCC TTG TCA CCT GTT CCC
Cys His Asn Ser Leu Ile Leu Pro Pro Phe Ser Leu Ser Pro Val Pro>

       920          930          940          950          960
ACC CTA GGA TGC CGC TCC CGC CGA GCG GTA CCG GTG GCG GTC TGG CTT
Thr Leu Gly Ser Arg Ser Arg Arg Ala Val Pro Val Ala Val Trp Leu>

          970          980          990         1000
                                               *
GTC TCC GCC CTG GCC ATG GGA GCC GGG GTG GCT GGC GGG ATT ACC GGC
Val Ser Ala Leu Ala Met Gly Ala Gly Val Ala Gly Gly Ile Thr Gly>

1010         1020         1030         1040         1050
TCC ATG TCC CTC GCC TCA GGA AAG AGC CTC TTA CAT GAG GTG GAC AAA
Ser Met Ser Leu Ala Ser Gly Lys Ser Leu Leu His Glu Val Asp Lys>

  1060         1070         1080         1090         1100
                                                       *
GAT ATT TCC CAA TTA ACT CAA GCA ATA GTC AAA AAC CAC AAA AAT CTA
Asp Ile Ser Gln Leu Thr Gln Ala Ile Val Lys Asn His Lys Asn Leu>

    1110         1120         1130         1140         1150
CTC AAA ATT GCG CAG TAT GCT GCC CAG AAC AGA CGA GGC CTT GAT CTC
Leu Lys Ile Ala Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu Asp Leu>

       1160         1170         1180         1190         1200
                                                             *
CTG TTC TGG GAG CAA GGA GGA TTA TGC AAA GCA TTA CAA GAA CAG TGC
Leu Phe Trp Glu Gln Gly Gly Leu Cys Lys Ala Leu Gln Glu Gln Cys>

          1210         1220         1230         1240
CGT TTT CTA AAT ATC ACT AAT TCC CAT GTC TCA ATA CTA CAA GAA AGA
Arg Phe Leu Asn Ile Thr Asn Ser His Val Ser Ile Leu Gln Glu Arg>

1250         1260         1270         1280         1290
CCC CCC CTT GAG AAT CGA GTC CTG ACT GGC TGG GGC CTT AAC TGG GAC
Pro Pro Leu Glu Asn Arg Val Leu Thr Gly Trp Gly Leu Asn Trp Asp>
```

Fig. 1 (cont'd.)

```
1300          1310          1320          1330          1340
 *
CTT GGC CTC TCA CAG TGG GCT CGA GAG GCC TTA CAA ACT GGA ATC ACC
Leu Gly Leu Ser Gln Trp Ala Arg Glu Ala Leu Gln Thr Gly Ile Thr>

   1350          1360          1370          1380          1390
CTT GTT GCG CTA CTC CTT CTT GTT ATC CTT GCA GGA CCA TGC ATC CTC
Leu Val Ala Leu Leu Leu Leu Val Ile Leu Ala Gly Pro Cys Ile Leu>

      1400          1410          1420          1430          1440
       *
CGT CAG CTA CGA CAC CTC CCC TCG CGC GTC AGA TAC CCC CAT TAC TCT
Arg Gln Leu Arg His Leu Pro Ser Arg Val Arg Tyr Pro His Tyr Ser>

          1450          1460
CTT ATA AAA CCT GAG TCA TCC CTG TAA 3'
Leu Ile Lys Pro Glu Ser Ser Leu ***>
```

FIG. 1 (cont'd.)

```
          9          18          27          36          45          54
ATG GGT AAT GTT TTC TTC CTA CTT TTA TTC AGT CTC ACA CAT TTT CCA CTA GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Met Gly Asn Val Phe Phe Leu Leu Leu Phe Ser Leu Thr His Phe Pro Leu Ala

          63          72          81          90          99         108
CAG CAG AGC CGA TGC ACA CTC ACG ATT GGT ATC TCC TCC TAC CAC TCC AGC CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gln Gln Ser Arg Cys Thr Leu Thr Ile Gly Ile Ser Ser Tyr His Ser Ser Pro

         117         126         135         144         153         162
TGT AGC CCA ACC CAA CCC GTC TGC ACG TGG AAC CTC GAC CTT AAT TCC CTA ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Ser Pro Thr Gln Pro Val Cys Thr Trp Asn Leu Asp Leu Asn Ser Leu Thr

         171         180         189         198         207         216
ACG GAC CAA CGA CTA CAC CCC CCC TGC CCT AAC CTA ATT ACT TAC TCT GGC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Asp Gln Arg Leu His Pro Pro Cys Pro Asn Leu Ile Thr Tyr Ser Gly Phe

         225         234         243         252         261         270
CAT AAG ACT TAT TCC TTA TAC TTA TTC CCA CAT TGG ATA AAA AAG CCA AAC AGA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Lys Thr Tyr Ser Leu Tyr Leu Phe Pro His Trp Ile Lys Lys Pro Asn Arg

         279         288         297         306         315         324
CAG GGC CTA GGG TAC TAC TCG CCT TCC TAC AAT GAC CCT TGC TCG CTA CAA TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gln Gly Leu Gly Tyr Tyr Ser Pro Ser Tyr Asn Asp Pro Cys Ser Leu Gln Cys

         333         342         351         360         369         378
CCC TAC TTG GGC TGC CAA GCA TGG ACA TCC GCA TAC ACG GGC CCC GTC TCC AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Tyr Leu Gly Cys Gln Ala Trp Thr Ser Ala Tyr Thr Gly Pro Val Ser Ser

         387         396         405         414         423         432
CCA TCC TGG AAG TTT CAT TCA GAT GTA AAT TTC ACC CAG GAA GTC AGC CAA GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Ser Trp Lys Phe His Ser Asp Val Asn Phe Thr Gln Glu Val Ser Gln Val

         441         450         459         468         477         486
TCC CTT CGA CTA CAC TTC TCT AAG TGC GGC TCC TCC ATG ACC CTC CTA GTA GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Leu Arg Leu His Phe Ser Lys Cys Gly Ser Ser Met Thr Leu Leu Val Asp

         495         504         513         522         531         540
GCC CCT GGA TAT GAT CCT TTA TGG TTC ATC ACC TCA GAA CCC ACT CAG CCT CCA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ala Pro Gly Tyr Asp Pro Leu Trp Phe Ile Thr Ser Glu Pro Thr Gln Pro Pro

         549         558         567         576         585         594
CCA ACT TCT CCC CCA TTG GTC CAT GAC TCC GAC CTT GAA CAT GTC CTA ACC CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Pro Thr Ser Pro Pro Leu Val His Asp Ser Asp Leu Glu His Val Leu Thr Pro

         603         612         621         630         639         648
TCC ACG TCC TGG ACG ACC AAA ATA CTC AAA TTT ATC CAG CTG ACC TTA CAG AGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ser Thr Ser Trp Thr Thr Lys Ile Leu Lys Phe Ile Gln Leu Thr Leu Gln Ser
```

FIG. 2

```
        657         666         675         684         693         702
ACC AAT TAC TCC TGC ATG GTT TGC GTG GAT AGA TCC AGC CTC TCA TCC TGG CAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Thr Asn Tyr Ser Cys Met Val Cys Val Asp Arg Ser Ser Leu Ser Ser Trp His

        711         720         729         738         747         756
GTA CTC TAC ACC CCC AAC ATC TCC ATT CCC CAA CAA ACC TCC TCC CGA ACC ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Val Leu Tyr Thr Pro Asn Ile Ser Ile Pro Gln Gln Thr Ser Ser Arg Thr Ile

        765         774         783         792         801         810
CTC TTT CCT TCC CTT GCC CTG CCC GCT CCT CCA TCC CAA CCC TTC CCT TGG ACC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Phe Pro Ser Leu Ala Leu Pro Ala Pro Pro Ser Gln Pro Phe Pro Trp Thr

        819         828         837         846         855         864
CAT TGC TAC CAA CCT CGC CTA CAG GCG ATA ACA ACA GAT AAC TGC AAC AAC TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Cys Tyr Gln Pro Arg Leu Gln Ala Ile Thr Thr Asp Asn Cys Asn Asn Ser

        873         882         891         900         909         918
ATT ATC CTC CCC CCT TTT TCC CTC GCT CCC GTA CCT CCT CCG GCG ACA AGA CGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Ile Ile Leu Pro Pro Phe Ser Leu Ala Pro Val Pro Pro Pro Ala Thr Arg Arg

        927         936         945         954         963         972
CGC CGT GCC GTT CCA ATA GCA GTG TGG CTT GTC TCC GCC CTA GCG GCC GGA ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Arg Ala Val Pro Ile Ala Val Trp Leu Val Ser Ala Leu Ala Ala Gly Thr

        981         990         999        1008        1017        1026
GGT ATC GCT GGT GGA GTA ACA GGC TCC CTA TCT CTG GCT TCC AGT AAA AGC CTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gly Ile Ala Gly Gly Val Thr Gly Ser Leu Ser Leu Ala Ser Ser Lys Ser Leu

       1035        1044        1053        1062        1071        1080
CTC CTC GAG GTT GAC AAA GAC ATC TCC CAC CTT ACC CAG GCC ATA GTC AAA AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Leu Glu Val Asp Lys Asp Ile Ser His Leu Thr Gln Ala Ile Val Lys Asn

       1089        1098        1107        1116        1125        1134
CAT CAA AAC ATC CTC CGG GTT GCA CAG TAT GCA GCC CAA AAT AGA CGA GGA TTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
His Gln Asn Ile Leu Arg Val Ala Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu

       1143        1152        1161        1170        1179        1188
GAC CTC CTA TTC TGG GAA CAA GGG GGT TTG TGC AAG GCC ATA CAG GAG CAA TGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Asp Leu Leu Phe Trp Glu Gln Gly Gly Leu Cys Lys Ala Ile Gln Glu Gln Cys

       1197        1206        1215        1224        1233        1242
TGC TTC CTC AAC ATC AGT AAC ACT CAT GTA TCC GTC CTC CAG GAA CGG CCC CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Cys Phe Leu Asn Ile Ser Asn Thr His Val Ser Val Leu Gln Glu Arg Pro Pro

       1251        1260        1269        1278        1287        1296
CTT GAA AAA CGT GTC ATC ACC GGC TGG GGA CTA AAC TGG GAT CTT GGA CTG TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Glu Lys Arg Val Ile Thr Gly Trp Gly Leu Asn Trp Asp Leu Gly Leu Ser

       1305        1314        1323        1332        1341        1350
CAA TGG GCA CGA GAA GCC CTC CAG ACA GGC ATA ACC ATT CTC GCT CTA CTC CTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Gln Trp Ala Arg Glu Ala Leu Gln Thr Gly Ile Thr Ile Leu Ala Leu Leu Leu
```

FIG. 2 (cont'd.)

```
      1359        1368        1377        1386        1395        1404
CTC GTC ATA TTG TTT GGC CCC TGT ATC CTC CGC CAA ATC CAG GCC CTT CCA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Leu Val Ile Leu Phe Gly Pro Cys Ile Leu Arg Gln Ile Gln Ala Leu Pro Gln

      1413        1422        1431        1440        1449        1458
CGG TTA CAA AAC CGA CAT AAC CAG TAT TCC CTT ATC AAC CCA GAA ACC ATG CTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Arg Leu Gln Asn Arg His Asn Gln Tyr Ser Leu Ile Asn Pro Glu Thr Met Leu

TAA 3'
---
***
```

FIG. 2 (cont'd.)

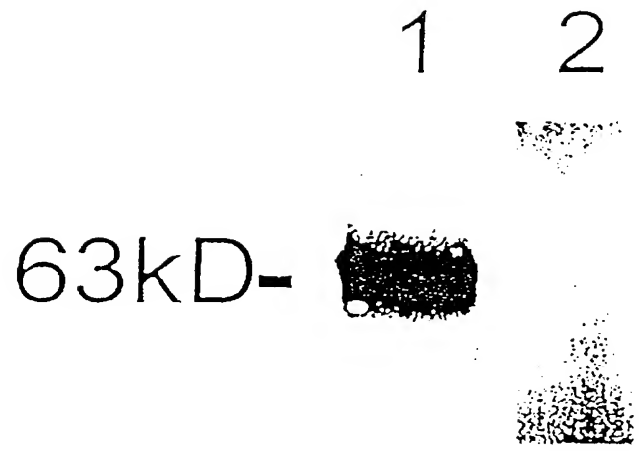

FIG. 3

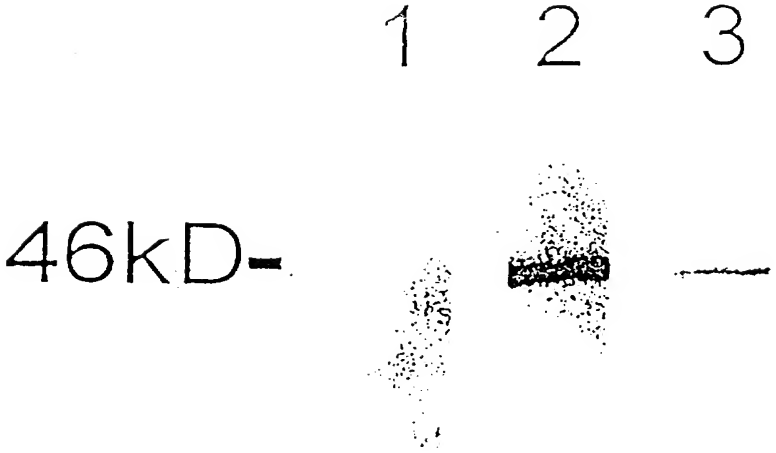

FIG. 4

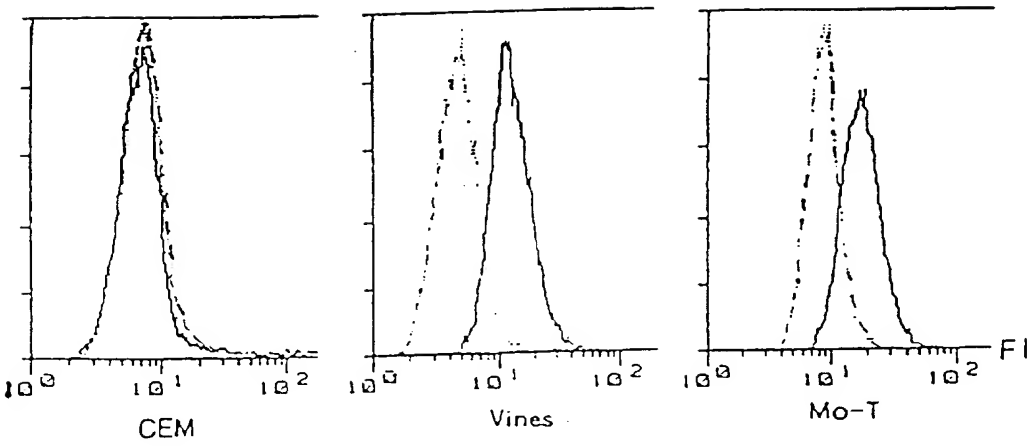

FIG. 5

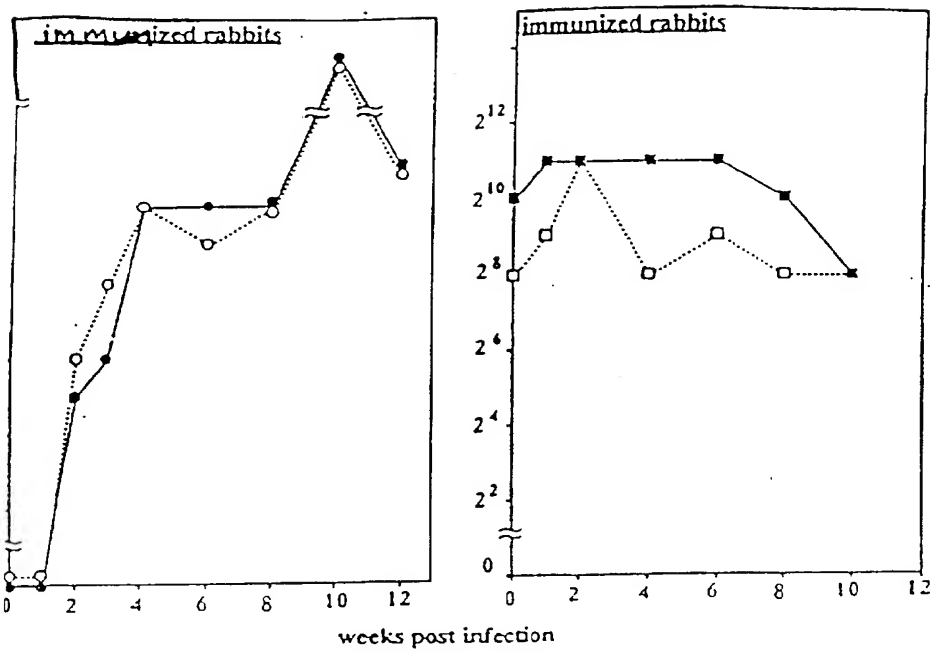
immunized rabbits
immunized rabbits
2^{12}
2^{10}
2^{8}
2^{6}
2^{4}
2^{2}
0
0 2 4 6 8 10 12
0 2 4 6 8 10 12
weeks post infection

FIG. 6A

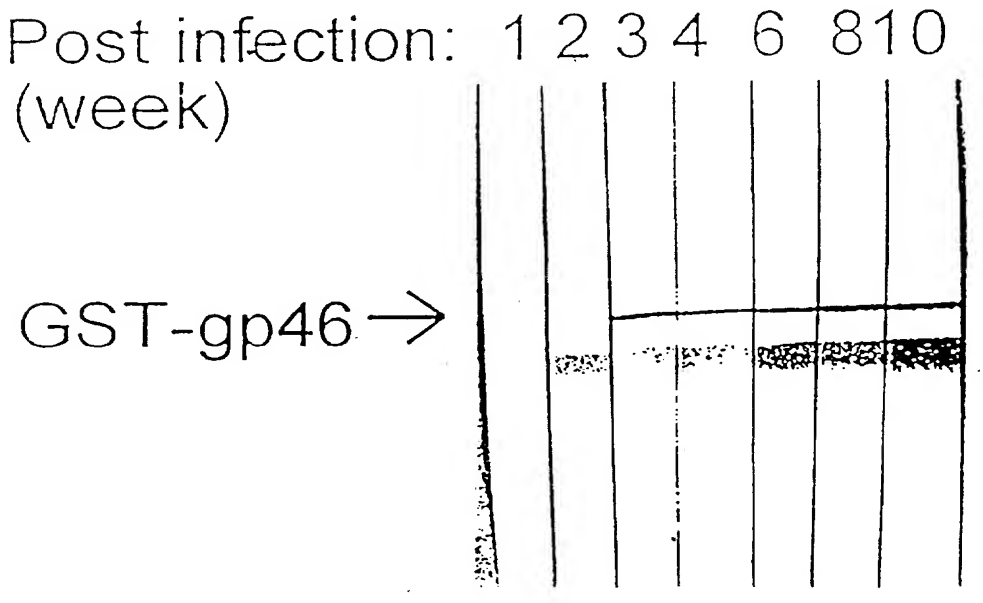
Post infection: 1 2 3 4 6 8 10
(week)
GST-gp46 →

FIG. 6B

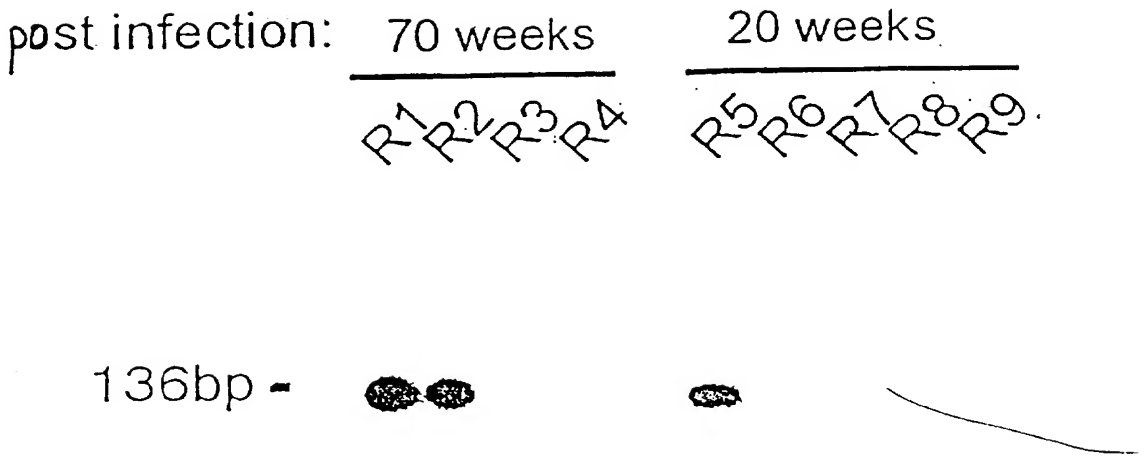
post infection: 70 weeks 20 weeks
R1 R2 R3 R4 R5 R6 R7 R8 R9
136bp

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12776

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/21, 38/04
US CL : 424/207.1, 89; 530/325, 326, 327, 328, 329, 350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/207.1, 89; 530/325, 326, 327, 328, 329, 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, AIDSLINE, SCISEARCH, BIOSIS, EMBASE
search terms: HTLV, human T cell lymphotropic virus, vaccaine, env gp63, glycopritein, recombinant

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	ARP. Expression and immunogenicity of the Entire Human T Cell Leukemia Virus Type I envelope protien produced in a baculovirus. Journal of General Virology. 1993. Vol. 74. pages 211-222, especially pages 212-213.	2, 7-9, -------- 4, 14, 16, 18, 22-24
X ------ Y	US 5,516,632 A (PALKER et al.) 14 May 1996, abstract, Example 6.	1-3, 5, 7, 9 -------- 4, 6, 10, 13, 15, 17, 19-21
X	SODROSKI. Sequence of the Envelope Glycoprotein Gene of Type II Human T lymphotropic Virus. July 1984. Science. Vol. 225. pages 421-424, especially abstract.	11, 12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 SEPTEMBER 1997	Date of mailing of the international search report 31 OCT 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer IW for BRETT L NELSON Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/12776

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,743,678 A (ESSEX et al.) 10 May 1988, column 3, lines 35-50.	6, 10, 20, 23